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UTILITY PATENT APPLICATION TRANSMITTAL <small>(Only for new nonprovisional applications under 37 CFR 1.53(b))</small>		Attorney Docket No. 710395.90010
		First Inventor or Application Identifier Richard L. Atkinson
		Title IRAL OBESITY METHODS AND COMPOSITIONS
		Express Mail Label No. E290771147US

09/19/00
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APPLICATION ELEMENTS <small>See MPEP Chapter 600 concerning utility patent application contents.</small>	ADDRESS TO: Commissioner for Patents Box Patent Application Washington, D.C. 20231
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1 <input checked="" type="checkbox"/> Fee transmittal Form <small>(Submit an original and a duplicate for fee processing)</small>	6. <input type="checkbox"/> Microfiche Computer Program (Appendix)
2 <input checked="" type="checkbox"/> Specification <small>[Total 21]</small> <small>(preferred arrangement set forth below)</small> <ul style="list-style-type: none">- Descriptive title of the invention- Cross References to Related Applications- Statement Regarding Fed Sponsored R&D- Reference to Microfiche Appendix- Background of the Invention- Brief Summary of the Invention- Brief Description of the Drawings (if filed)- Detailed Description- Claim(s)- Abstract of the Disclosure	7. Nucleotide and/or Amino Acid Sequence Submission <small>(if applicable, all necessary)</small> <input type="checkbox"/> Computer readable Copy <input checked="" type="checkbox"/> Paper Copy (identical to computer copy) <input type="checkbox"/> Statement Verifying identity of above
3 <input type="checkbox"/> Drawing(s) (35 USC 113) <small>[Total Sheets]</small>	ACCOMPANYING APPLICATION PARTS 8 <input type="checkbox"/> Assignment Papers (cover sheet & documents) 9 <input type="checkbox"/> 37 CFR 3.73(b) Statement <input type="checkbox"/> Power of Attorney <small>(where there is an assignee)</small> 10 <input type="checkbox"/> English Translation Document <small>(if applicable)</small> 11 <input type="checkbox"/> Information Disclosure Statement (IDS)/PTO-1449 <input type="checkbox"/> Copies of IDS Citations 12 <input type="checkbox"/> Preliminary Amendment 13 <input checked="" type="checkbox"/> Return receipt postcard (MPEP 503) <small>(Should be specifically itemized)</small> 14 <input type="checkbox"/> *Small Entity Statement(s) <input checked="" type="checkbox"/> Statement filed in prior application <small>Status still proper and desired</small> 15 <input type="checkbox"/> Certified copy of priority Document(s) <small>(if foreign priority is claimed)</small> 16 <input checked="" type="checkbox"/> Other: Copy of Revocation, Power of Attorney... <small>* A new statement is required to pay small entity fees, except where one has been filed in a prior application and is being relied upon</small>
4. Oath or Declaration <small>[Total Pages 3]</small> a. <input type="checkbox"/> Newly executed (original or copy) b. <input checked="" type="checkbox"/> Copy from prior Application (37 CFR 1.63(d)) <small>(for continuation/divisional with Box 17 completed)</small> [Note Box 5 below] i. <input type="checkbox"/> DELETION OF INVENTOR(S) <small>Signed Statement attached deleting inventor(s) named in prior application, see 37 CFR 1.63(d)(2) and 1.33(b).</small>	
5 <input type="checkbox"/> Incorporation By Reference (useable if Box 4b is checked) <small>The entire disclosure of the prior application from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference herein.</small>	
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VIRAL OBESITY METHODS AND COMPOSITIONS

RELATED APPLICATION

For this Application priority of United States Provisional Patent
5 Application Serial No. 60/042,942, filed April 4, 1997, is claimed.

TECHNICAL FIELD

This invention concerns obesity in humans caused by viruses and
methods and compositions for diagnosing, treating and preventing this
10 disease.

The invention also concerns methods and compositions for reducing
levels of triglycerides and cholesterol in humans.

More particularly, the invention concerns methods and compositions
for diagnosing whether obesity in a human is caused by a virus or whether a
15 person is susceptible to becoming obese because of having been infected with
and obesity-causing virus, methods for testing or screening body fluids (*e.g.*,
donated human blood) for the presence of obesity-causing viruses, methods
for treating and preventing viral obesity in humans, methods for preparing
vaccine compositions for treating and preventing viral obesity in humans,
20 such vaccine compositions themselves, and viruses which cause viral obesity
in humans.

Further, the invention concerns methods for reducing serum levels of
triglycerides and cholesterol, including low-density-lipoprotein-associated
cholesterol, in humans by administration thereto of a virus which causes viral
25 obesity in humans.

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BACKGROUND OF THE INVENTION

Obesity is a serious disease of humans. A person is clinically obese if he or she has excess adipose tissue. More particularly, for purposes of this application, a person is obese if the person's body mass index equals or exceeds 27 kg/m² and the person has excess adipose tissue. In the medical arts, the quantity of adipose tissue that is "excessive" is not well defined; but certainly greater than 25% of body weight as fat in a male and greater than 30% of body weight as fat in a female would be excessive.

Obesity has a number of known and suspected etiologies. See A. Sclafani, "Animal Models of Obesity: Classification and Characterization," *Int. J. Obesity* 8, 491-508 (1984); G. A. Bray, "Classification and Evaluation of the Obesities," *Med. Clin. N. Am.* 73, 161-184 (1989).

There is a strong positive correlation of increased body weight with elevated serum levels of triglycerides (TG) and cholesterol (CHOL), including low-density-lipoprotein-associated cholesterol (LDL-CHOL). Thus, obesity, in its known forms, is often associated with elevated serum levels of these substances.

The prevalence of obesity is increasing worldwide. The prevalence in the US population remained essentially constant, at about 25 %, from 1960 to 1980. The prevalence in the US population increased between 1980 and 1990 to more than 33 % and continues to increase. About 90 million people in the US are obese today. Similar statistics prevail in the rest of the world.

Obesity, in people who have the disease, is associated with physical, psychological, and social problems. Complications of obesity include, among others, diabetes mellitus, hypertension, hyperlipoproteinemia, cardiac diseases (atherosclerotic disease, congestive heart failure), pulmonary diseases (sleep apnea, restrictive lung disease), cerebrovascular accidents,

cancers (breast, uterus, colon, prostate), gall bladder disease (stones, infection), toxemia during pregnancy, risks during surgery (pneumonia, wound infection, thrombo-phlebitis), gout, decreased fertility, degenerative arthritis, and early mortality.

5 Psychological complications of obesity include poor self-image and poor body-image. These complications are due in part to the fact that obesity is socially disfavored.

10 The fact that obesity is socially disfavored also presents social problems for obese people. Among these is discrimination in jobs, education and marriage.

 Clearly, there is a need for methods to treat or prevent obesity. Effective treatment or preventative methods likely vary among the obese depending on the etiology of the obesity which an individual has.

15 Thus, there is a need to understand further the various etiologies of obesity. Such understanding will lead to methods and compositions to effectively treat or prevent the disease.

20 Further understanding of the etiologies of obesity also will lead to reduction in the prevalence of the social stigma associated with the disease, as it will allow the public at large to understand better that obesity is a disease which might afflict anyone and from which people do not choose to suffer. Such understanding also will allow obese persons to be convinced that they are unwilling victims of a disease, to understand through various diagnostic tests based on understanding of etiologies of the disease what the underlying cause of their obesity is, and in some cases to learn how to

25 effectively treat the disease. Reduction in the prevalence of the social stigma associated with obesity and increased understanding among the obese concerning the disease will diminish the psychological complications and

social problems which affect obese persons because of the disease.

Still further, understanding of the etiologies underlying obesity and the corresponding recognition that obesity is a disease eventually will lead medical insurance companies, which now at least in the United States typically do not recognize the condition as a disease, to recognize it as such and reimburse persons for diagnosis and treatment of it in the same way that the companies now do so for conditions that have long been recognized as diseases.

There has been speculation that one etiology of obesity in humans might be viral. A. Sclafani, *supra*. However, there has been no convincing evidence to support this speculation. Heretofore no virus has been identified as a cause of the disease in humans.

SUMMARY OF THE INVENTION

We have discovered that some obesity in humans is caused by viruses.

We have identified a human virus which infects or has infected about 15 - 20 % of obese persons in the general population.

Further we have discovered that humans who suffer from viral obesity (*i.e.*, obesity caused by a virus) have, on the average, significantly lower TG, CHOL and LDL-CHOL levels than persons who suffer from obesity that is not viral. In fact, the average TG, CHOL and LDL-CHOL levels of persons with viral obesity are within the normal ranges for persons who are not obese.

Our discoveries are bases for the various methods and compositions of the invention as described below in detail.

DETAILED DESCRIPTION OF THE INVENTION

We have found that certain adenoviruses, but not all adenoviruses, that infect humans cause obesity in humans that are infected therewith and, at the same time, lower the average TG, CHOL and LDL-CHOL levels in those who are infected.

Screening of human adenoviruses for those that cause obesity and "reduced cholesterol levels" (by which is meant herein TG, CHOL and LDL-CHOL levels that in an infected population are reduced on the average compared to a control population of obese individuals who are not infected with a virus of interest) in humans is done by methods known in the art. Namely, in a preferred method, first by antibody-screening a group of persons (*e.g.*, more than about 10, preferably more than about 50, and more preferably more than about 100) is identified who have been infected by a particular strain of adenovirus and who are both obese and have, as a group, a mean cholesterol level that is reduced, as described above in this paragraph. Such an adenovirus strain is a "candidate virus." Then, to determine whether a candidate virus is one that causes obesity and reduces cholesterol levels, the virus is tested by methods recognized in the art, by infecting a group of animals such as chickens, mice, rats or monkeys, with the virus in order to test the virus for the ability to cause obesity and reduce cholesterol levels on the average in the group of infected animals. A candidate virus, which on testing in a group of animals does cause obesity and reduce the average cholesterol level in the group, is one which causes obesity and reduces cholesterol levels in humans and provides a basis for developing methods and compositions for diagnosing and treating obesity, screening for obesity-causing virus, and reducing cholesterol levels, in humans as

understood in the art and described more fully hereinafter. These methods and compositions using any obesity-causing, cholesterol reducing adenovirus that infects humans are encompassed by the present invention.

By applying the foregoing methods, we have found that human
5 adenoviruses Ad-2 and Ad-31, which are available from the American Type Culture Collection ("ATCC"), do not cause obesity or reduce cholesterol levels in humans but that adenovirus Ad-36, which is also available from the ATCC, and a variant thereof that we have isolated, adenovirus Ad-36p, do do so.

10 Thus, in one of its aspects, the invention is substantially isolated human adenovirus Ad-36P.

"Substantially isolated" is defined herein with reference to a sample of viruses that is in an *in vitro* system such as a culture, a plate on which culture is grown, a suspension in buffer or culture medium, a band on a gel,
15 a pellet resulting from centrifugation of a suspension comprising virus, or the like and that comprises one or more variants of human adenovirus strain Ad-36 as available from the ATCC (Rockville, Maryland, USA) under accession no. VR-913.

We have found that strain Ad-36 as obtained from the ATCC
20 comprises a number of variants of the virus. One of these variants is adenovirus Ad-36P. Thus, by "substantially isolated" is meant that the virus is in an *in vitro* system as described above and, in the system, the ratio of the number of particles of Ad-36P to the number of particles of all variants of strain Ad-36 is greater than such ratio in strain Ad-36 obtainable from the
25 ATCC under accession no. VR-913.

Preferably, of course, a sample of Ad-36P in an *in vitro* system will consist essentially of virus particles of that variant alone, as might be

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obtained for example by standard passaging at least three times of strain Ad-36 as obtained from the ATCC.

Strain Ad-36p is readily available to the art, as it can be isolated from some humans who are obese and who have reduced cholesterol levels (as indicated above, in comparion with obese persons who are not infected with an obesity-causing, cholesterol-level-reducing adenovirus).

Herein below we present the nucleotide sequence of cDNA that encodes the fiber protein of strain Ad-36p. This sequence further serves to identify the strain.

We have discovered adenovirus Ad-36P to be surprisingly sensitive, and specifically more sensitive than Ad-36 as obtained from culture obtained from the ATCC, in detecting serum antibody that reacts with Ad-36.

Adenovirus Ad-36P, like Ad-36, when it infects a person, causes the person to become obese with an obesity characterized by TG, CHOL and LDL-CHOL levels that, on the average in the infected population, are reduced compared to obese persons who are not infected with obesity-causing virus and in fact, in some obese and infected individuals (typically ones who are not severely obese) are not elevated above the normal levels in persons who are not obese.

In another of its aspects, the invention is an anti-obesity vaccine which comprises as the active ingredient a composition selected from the group consisting of: (i) killed virus and inactivated live virus, wherein the corresponding live (non-inactivated) virus infects humans, and causes obesity and reduced cholesterol levels in individuals infected with the virus; (ii) a coat protein of the coat of such a virus; (iii) an immunogenic peptide with from about 6 to about 30 amino acids in a sequence which comprises the sequence of an epitope of such a coat protein; (iv) nucleic acid (DNA or

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RNA) with a sequence which encodes such a coat protein or such an immunogenic peptide; and (v) a non-pathogenic virus, such as a vaccinia virus or fowl-pox virus, which is genetically modified to have a modified coat protein which comprises in immunogenic position and orientation a segment with the sequence of a coat protein of a virus which infects humans and causes obesity and reduced cholesterol in infected individuals or a sequence of a peptide of from about 6 to about 30 amino acids in a sequence which comprises the sequence of an epitope of a virus which infects humans and causes obesity and reduced cholesterol in infected individuals.

10 The various active ingredients in the vaccines of the invention are either immunogenic *per se* or provide *in vivo* in a vaccinated person protein or peptide which is immunogenic. Peptides of 6 - 30 amino acids, to be rendered acceptably immunogenic, might require modification by any of various techniques known in the art (*e.g.*, conjugation with a large protein).

15 The various vaccines in accordance with the invention are all prepared by methods known in the art.

From the adenoviruses which can be the bases for an anti-obesity vaccine according to the invention, because they infect humans and cause obesity and reduced cholesterol levels on the average in infected individuals (among these Ad-36p being the most preferred), hexon or fiber proteins, preferably the fiber protein, or segments of the fiber protein, as indicated above, can be used as the immunogen.

20 By the term "immunogenic," with reference to an anti-obesity vaccine of the invention, is intended capability to provoke in a person to whom the vaccine is administered an immune response that yields neutralizing antibodies against an obesity-causing, live virus that might infect the person after administration of the vaccine.

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The anti-obesity vaccines of the invention, wherein the immunogenic component is live, inactivated virus, killed virus, coat protein *per se*, epitope-comprising coat protein segment, or coat protein (or epitope-comprising segment thereof) provided with use of a non-pathogenic, genetically modified carrier virus such as a vaccinia virus or a fowl pox virus, are prepared using methods well known in the art. Thus, the vaccines will include carriers, excipients, adjuvants, antimicrobials, preservatives and the like as well understood in the art. Thus, in addition to the active ingredient, the vaccines will have suitable compositions, usually aqueous buffers, such as phosphate-buffered saline or the like, in which the active ingredient will be suspended along with, optionally, any of various immune-system stimulating adjuvants used in human vaccine preparations, antimicrobial compositions, and other compositions to stabilize the preparations. All compositions included with the vaccine preparation will be suitable for administration to humans. The vaccine preparation may be stored in lyophilized form and then combined with solution soon before administration. For oral administration, the vaccine preparation may be in solution, tablet or pill form optionally with an enteric coating as understood in the art. The concentration of active (immunogenic or immunogen-providing) component in solution with which it is administered typically will be between about 1 ng and about 1 mg/ml.

The anti-obesity vaccines of the invention will be administered intranasally, orally, or by injection intravenously, intramuscularly, subcutaneously or peritoneally.

Administration of the vaccines of the invention is to be under the guidance of a physician.

Appropriate dosing of the anti-obesity vaccine is well within the skill of medical practitioners and will depend on a number of factors including the age of the person being treated, the urgency of the person's developing protective immunity, the status of the person's immune system, and other factors known to the skilled. The vaccine typically will be administered in several steps in order to cause and maintain protective immunity against obesity-causing virus in the person being vaccinated. Thus, after the primary vaccination, there typically will be between one and about ten booster vaccinations separated by periods between about 1 week and 10 years.

A single dose of an anti-obesity vaccine of the invention (in solution form) will have a volume of between about 0.1 ml and 10 ml and, in any form, will have between about 1 ng and 10 mg of killed or inactivated obesity-causing virus, between about 1 ng and 10 mg of genetically modified, non-pathogenic virus, or between about 1 ng and 10 mg of coat protein (e.g., fiber protein) or 6-30 amino acid peptide (in its form as modified to be immunogenic).

An anti-obesity vaccine of the invention, wherein the active ingredient is nucleic acid, will also be a standard preparation for vaccines of that type. With vaccines of this type, the nucleic acid is not the immunogen but is expressed *in vivo* after administration of the vaccine as a peptide or protein which in turn is immunogenic. Vaccines of this type will be administered by techniques known in the art for such vaccines (e.g., intramuscular injection). Dosing will also be according to procedures known in the art to cause and maintain protective immunity against viral obesity in the vaccinated individual.

Note that an anti-obesity vaccine according to the invention may include active ingredients based on more than one obesity-causing virus (or

the coat protein (e.g. fiber protein) or epitopic segments of the coat protein thereof).

In yet another aspect, the invention is a method of preventing obesity caused by a virus in a human susceptible thereto which comprises
5 administering to the human an amount of an anti-obesity vaccine of the invention that is effective to raise and maintain a protective immune response against an obesity-causing adenovirus.

In still another aspect, the invention entails a method of reducing the serum levels of triglyceride, (total) cholesterol and low-density-lipoprotein-associated cholesterol in a person with above normal serum levels thereof
10 which comprises administering to the person an amount effective for such purpose of a live virus which infects humans and causes obesity in infected individuals.

"Normal" serum levels are as follows: triglycerides (TG): less than 150
15 mg/dL; (total) cholesterol (CHOL): less than 200 mg/dL; low-density-lipoprotein-associated cholesterol (LDL-CHOL): less than 130 mg/dL.

In a preferred application of this method of the invention, the TG, CHOL and LDL-CHOL levels would all be reduced to normal levels.

This method of the invention could especially advantageously be
20 applied to persons who are at very high risk of complications or death from atherosclerotic disease or congestive heart failure because of high serum levels of triglyceride (hypertriglyceridemia) or cholesterol (hypercholesterolemia).

The method is carried out by administering the live virus, or a more
25 preferably a fragment of the virus such as the E1A fragment which is a well known fragment of adenoviruses, in a conventional medium that is acceptable for administration to humans, to the person being treated in one

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or more doses sufficient to infect the person with the virus. The administration can be intranasal, oral, or by injection intravenously, subcutaneously, intramuscularly, or peritoneally.

Among the live viruses that can be employed in this method of the invention are obesity-causing, cholesterol-level-reducing, human adenoviruses, such as Ad-36 and Ad-36P, and the E1A fragments thereof. E1A fragments may be delivered intracellularly in a person being treated by methods known in the art using innocuous viruses, such as vaccinia viruses or retroviruses (modified so as to be non-infectious). The innocuous viruses used in accordance with the invention will have genomes that are modified to include DNA that encodes the E1A fragment in a position where the fragment will be expressed in cells of the individual that have been infected with the innocuous virus. The innocuous virus, so modified, will be administered to an individual being treated so as to infect the individual (and cells of the individual) with the virus. As described hereinabove, as known in the art, DNA itself (in an appropriate solution) which comprises a segment that encodes the E1A fragment may be injected directly into an individual being treated to provide E1A intracellularly in the individual.

In still another aspect, the invention entails methods for diagnosing whether a non-obese person is at risk of acquiring viral obesity, a method for diagnosing whether an obese person suffers from viral obesity, and a method of screening body fluids or organs and tissues (especially donated blood or donated organs or tissues) for the presence of obesity-causing adenoviruses. These methods of the invention comprise analyzing, by any conventional immunoanalytical or nucleic-acid probe hybridization based procedure, a sample of blood, other body fluid, feces, tissue or organ for the presence of antibody reflecting infection with the virus, the presence of a protein (e.g., a

fiber protein) characteristic of the presence of the virus, or the presence of a nucleic acid fragment characteristic of the presence of the virus.

As understood in the art, a person whose blood, other body fluids, feces, or tissues or organs has been or is infected with the virus but who is not obese will be at greater risk than a person who has not been or is not so infected of becoming obese on account of infection by the obesity-causing virus and might be treated early to slow or prevent the onset of the disease. Similarly, an obese person whose blood, other body fluids, feces, or tissues or organs has been or is infected with the virus can ascribe his or her obesity at least in part to infection with the obesity-causing virus and, for example, might suffer less severe psychological or social trauma by understanding that he or she is suffering from an obesity that is a conventional disease or may demand reimbursement from his or her insurance company for having a type of obesity that is a conventional disease, caused by infection with a disease-causing virus. Also, the risk of spreading viral obesity to persons who are not infected with obesity-causing virus would be drastically reduced by screening of blood by blood banks, of blood that is donated for use by others than the donees, or screening of tissues or organs by tissue and organ banks or collecting agencies, of tissues or organs donated for transplantation, for the presence, past or present, of obesity-causing virus so that such blood, tissues or organs will not be used after having been donated.

The immunoanalytical techniques that might be used in these methods are standard virus neutralization assay techniques or enzyme immunoassay techniques made possible by the availability of obesity-causing viruses identified and made available by the present invention. Antibodies against these viruses or fragments thereof (e.g., fiber protein or fragments thereof), or proteins (or fragments thereof) from these viruses for use in these

immunoassay techniques can be prepared by conventional techniques well known in the art.

Similarly, the nucleic acid probe hybridization assay techniques used in these methods of the invention will be standard techniques (optionally after
5 amplification of DNA or RNA extracted from a sample of blood, other body fluid, feces, tissue or organ) using nucleic acid probes (and primers if amplification is employed) made available by the obesity-causing viruses identified and made available by the present invention. The sequences of nucleic acids characteristic of these viruses can be determined by standard
10 techniques once the viruses are conventionally isolated, and probes and primers that are specific for the viruses and that provide the basis for nucleic acid probes and primers that can be used in nucleic acid based assays for the viruses are prepared using conventional techniques on the basis of the sequences.

15 As the skilled will understand, more than one strain of obesity-causing virus may be tested for simultaneously in an immunological or nucleic acid-based assay method for testing for virus in accordance with the invention and kits may be assembled to facilitate carrying out the methods for a particular virus or a plurality of them.

20 Further details on carrying out the invention are now provided in the following examples, which are illustrative and should not be construed to limit the scope of the invention.

EXAMPLE 1

Serum cholesterol (total)(TG) is determined using fasting serum, 10 microliter samples, in a cholesterol-oxidase-peroxidase method employing a kit from Sigma Chemical Co., St. Louis, MO, USA (Cat No. 352-500P).

5 Serum triglyceride (TG) is determined using fasting serum, 10 microliter samples, in a glycerol-3-phosphate peroxidase method employing a kit from Sigma Chemical Co. (Cat. No. 339-50).

Serum high-density-lipoprotein-associated cholesterol (HDL-CHOL) is determined using fasting serum with a kit from Sigma Chemical Co.

10 Low-density-lipoprotein-associated cholesterol (LDL-CHOL) is determined using the equation

$$\text{LDL-CHOL} = (\text{CHOL}) - (\text{HDL-CHOL}) - (\text{TG}/5).$$

EXAMPLE 2

15 Ad-36 virus was obtained from the ATCC (American Type Culture Collection), Accession No. VR-913. The virus was grown in A549 bronchial human carcinoma cells (Wisconsin State Laboratory of Hygiene, University of Wisconsin, Madison, Wisconsin, USA). Plaques were grown, and a single plaque was removed and used to reinfect a fresh batch of A549 cells.

20 Plaques of this second passage were grown and again a single plaque was picked and used to reinfect another fresh batch of A549 cells. The resulting virus is Ad-36P.

In virus neutralization assays of 5 human sera known to have antibodies to Ad-36 (ATCC VR-913), Ad-36p showed a 2- to 4-fold greater titer for neutralizing antibodies than did Ad-36. This demonstrates the greater sensitivity of Ad-36p, as compared to Ad-36, in assaying serum for antibody against obesity-causing adenoviruses.

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EXAMPLE 3

Minimum Essential Media Eagle (MEM)(Sigma Chemical Co. Cat. No. M-0643) with non-essential amino acids, Earle's salts, l-glutamine, 10 % fetal bovine serum and 2.9 % sodium bicarbonate (v/v), pH 7.4, is used for growing A549 cells. 5 to 10 microliters of virus stock solution is mixed with 8 ml of the medium and the mixture is pipetted into flasks with growing cells. The flasks are incubated for 1 h at 37 deg. C while shaking gently every 15 min. After 1 h, the medium is removed and replaced with fresh medium, The flasks are then incubated at 37 deg. C. The cells are grown over 7 to 8 days, until 90 % of the cells show CPE. The cell material and medium are then centrifuged at 1000 rpm for 15 min to eliminate cell debris. The supernatant. with virus (*e.g.*, Ad-36 or Ad-36P), is again centrifuged at 1000 rpm for 10 min and then aliquoted into 2 ml cryovials for storage at -70 deg. C until use.

EXAMPLE 4

Fiber protein is isolated from virus in a two step procedure. First, virus is purified from supernatant (see Example 3) using CsCl gradient centrifugation, following the procedure described by Graham and Ludvik in Chapter 11 of *Methods in Molecular Biology, Vol. 7: Gene transfer and Expression Protocols*, E. J. Murray, ed., The Humana Press, Inc., Clifton, N.J. (1991). Then the fiber protein is isolated from the virus electrophoretically following Oostrum and Burnett, *J. Virology*, 56, 439-448 (1985) and Maizel *et al.*, *Virology*, 36, 115-125 (1968).

The electrophoretic band of fiber protein (approx. mol. wt. 60,000 daltons) is cut from the gel and the fiber protein can be isolated from the band by standard techniques.

EXAMPLE 5

A virus neutralization assay (serum neutralization assay) is used to assay serum for antibody reactive with adenovirus in serum of test subjects.

Serum is thawed and heat-inactivated for 30 min. at 56 deg. C.

- 5 The assay is carried out in standard 96-well microtiter plates. Serial two fold dilutions (1:2 to 1:1024) are made with the medium that is the A549 growth medium described in Example 3 but lacks the fetal calf serum and sodium bicarbonate. 50 microliters of each dilution is added in duplicate to the wells of the plate. 50 microliters of virus suspension (100 TCID_{50}) is then
- 10 added to each well. (TCID_{50} is calculated by serially diluting viral stock solution and inoculating A549 cells with the dilutions to determine the reciprocal of the highest dilution of virus which causes CPE in 50 % of the material inoculated.) The plates are then incubated at 37 deg. C for 1 hr. Then 100 microliters of A549 cell suspension, containing approximately
- 15 20,000 cells, is added to each well and the plate is further incubated at 37 deg. C for 12 days. Crystal violet- ethanol is then added to each of the wells to fix and stain the cells and the plates are examined macroscopically for CPE;. The highest serum dilution with no CPE is the titer. Controls used in the procedure are wells with no virus and wells with virus but no serum. A
- 20 back titration is carried out to confirm that appropriate virus dilutions were used. Positive control is antisera to chicken adenovirus and human adenovirus. Presence of CPE with the virus and no CPE in the presence of serum is considered an indication of effective neutralization of the virus with antibody in serum, such that the serum is considered to have antibody against
- 25 the virus. A titer of 1:8 or greater is considered positive.

The foregoing assay was carried out on serum samples from 155 obese patients and 45 non-obese volunteers. 15 - 20 % of the obese patients were

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positive for antibody. All of these had TG, CHOL, and LDL-CHOL within the normal ranges for non-obese people. The remaining obese people (antibody-negative) had, on the average, TG, CHOL and LDL-CHOL levels above the normal ranges for non-obese people. None of the non-obese
 5 volunteers was positive for antibody. The non-obese volunteers had, on the average, TG, CHOL and LDL-CHOL levels that were in the normal ranges for non-obese people.

EXAMPLE 6

10 Ad-36P virus is killed by adding 42 microliters of 37 % formalin to 150 microliters of virus stock, then incubating the resulting composition at room temperature for 72 hours, and finally adding 15 microliters of 35 % sodium bisulfite. Confirmation that the virus was killed is carried out by inoculating a culture of A549 cells with the final solution and determining that
 15 virus does not grow in the culture.

EXAMPLE 7

Nucleic acid from adenovirus Ad-36p was isolated and sequenced by a standard sequencing method.

20 The cDNA sequence encoding the fiber protein is as follows
 (SEQ ID NO:1):

5'-

ATGTCAAAGAGGCTCCGGGTGGAAGATGACTTCAACCCCGTCT
 ACCCCTATGGCTACGCGCGGAATCAGAATATCCCCTTCCTCACT
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 20 ACCATATGATACCTCTTCTTTTACTTTCTCATATATCGCTCAAGA
 ATGA

EXAMPLE 8

The cDNA sequence of the Ad-36p genome was screened against
 25 all known cDNA sequences and two 25-base sequences and one 28-base
 sequence were found, all lying in the fiber-encoding sequence provided
 above in Example 7, that were unique to Ad-36p. These three sequences

are as follows:

SEQ ID NO:2: 5'-AGTTGAAACAGCAAGAGACTCAAAG

SEQ ID NO:3 5'-GGTACTGGATCAAGTGCACATGGAG

5

SEQ ID NO:4 5'-TTGAAACAGCAAGAGACTCAAAGCTAAC

Sequence 3 above was employed as a probe for AD-36p in a conventional nucleic acid probe hybridization assay of DNA isolated from four
10 chickens, two of which had been infected with the virus and became obese and two of which had not been infected and were not obese. DNA hybridizing to the probe was observed with only the DNA from the two infected chickens. The assay involved direct detection and was by capillary electrophoresis using laser-induced fluorescence for detection. More
15 particularly, a replaceable polyacrylamide matrix was employed in the electrophoretic separation and detection employed a dual system with 5'-labeling of the oligo and thiazole orange intercalator in the buffer system. See Kolestar *et al.*, J. Chromatography B, 697, 189-194 (1997).

The skilled will understand that probes, and primers when
20 amplification is also used, of between about 15 and 30 bases in length are advantageously employed to provide suitable specificity and sensitivity.

Amplification methods using PCR and variations thereof maybe employed, as well known in the art.

WHAT WE CLAIM IS:

1. Substantially isolated Ad-36p.

5 2. A method of determining whether an obese person is suffering from viral obesity which comprises analyzing a body fluid, feces, a sample of tissue or a sample of an organ from the person by an immunoanalytical or nucleic acid probe hybridization acid method to ascertain whether the person has been or is infected with an adenovirus that causes obesity and reduces cholesterol levels in humans.

10

3. The method according to Claim 2 wherein the substance analyzed is blood.

00619117-071900

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PTO/SB/01 (12-87)

Approved for use through 9/30/00. OMB 0651-0092
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)	Attorney Docket Number	10006-4		
	First Named Inventor	Atkinson		
	COMPLETE IF KNOWN			
	Application Number	09 / 056,153		
	Filing Date	April 6, 1998		
	Group Art Unit			
<input type="checkbox"/> Declaration Submitted with Initial Filing	OR	<input checked="" type="checkbox"/> Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)	Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

VIRAL OBESITY METHODS AND COMPOSITIONS

the specification of which (Title of the Invention)

☐ is attached hereto
OR

☒ was filed on (MM/DD/YYYY) April 6, 1998 as United States Application Number or PCT International

Application Number 09/056,153 and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
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☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	<input type="checkbox"/> Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.
60/042,942	April 4, 1997	

[Page 1 of 2]

Burden Hour Statement: This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

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U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

<input type="checkbox"/> Customer Number 		<input type="checkbox"/> Registered practitioner(s) name/registration number listed below	
Name	Registration Number	Name	Registration Number
William J. Scanlon	30,136		

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: ☐ Customer Number or Bar Code Label OR ☒ Correspondence address below

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Address	Suite 1, 616 South Ingersoll Street		
City	Madison	State	WI
ZIP	53703-3810		
Country	USA	Telephone	(608) 294-1141
Fax	(608) 294-1322		

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:		<input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name (first and middle if any)		Family Name or Surname	
Richard W. L. RLA 10/21/98		Atkinson	
Inventor's Signature	Richard W. Atkinson		Date
Residence: City	Fitchburg	State	WI
Country	USA		Citizenship
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Post Office Address			
City	Fitchburg	State	WI
ZIP	53575		Country
USA			

☒ Additional inventors are being named on the supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

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<input type="checkbox"/> Customer Number <input type="text"/> OR <input type="checkbox"/> Registered practitioner(s) name/registration number listed below		Place Customer Number Bar Code Label here	
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Name			
Address			
Address			
City	State	ZIP	
Country	Telephone	Fax	

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Additional Name of Signatory: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle if any)	Family Name or Surname
Nikhil V.	Dhurandhar

Inventor's Signature	<i>Nikhil V. Dhurandhar</i>	Date	Oct. 21 1998
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Residence: City	Madison	State	WI	Country	USA	Citizenship	India
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City	Madison	State	WI	ZIP	53711	Country	USA
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☐ Additional inventors are being named on the supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Richard L. Atkinson Date: _____, 1998
 Nikhil V. Dhurandhar

Serial No.: 09/056,153 Examiner: A. Salimi

Filed: April 6, 1998 Art Unit: 1643

Title: VIRAL OBESITY METHODS Our Ref: 710395.90010
 AND COMPOSITIONS

REVOCATION, POWER OF ATTORNEY AND
APPOINTMENT OF POWER OF ATTORNEY

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

The undersigned on behalf of Obetech LLC, the assignee of the complete interest of the above-identified patent application, hereby cancels all previously executed powers of attorney in this application and appoints as power of attorney, with full powers of substitution and appointment the following:

Nicholas J. Seay
Reg. 27,387
Quarles & Brady LLP
P O Box 2113
Madison, WI 53701-2113
(608) 251-5000

Please direct any further correspondence in regard to this application to the above attorney.

Respectfully submitted

OBETECH LLC

Dated: June 16, 1999

By: Richard L. Atkinson
Name: Richard L. Atkinson, MD
Title: Principal

SEQUENCE LISTING

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Dhurandhar, Nikhil V.

<120> Viral Obesity Methods and Compositions

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